(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 894 496 A1

(12) EUROPEAN PATENT APPLICATION

published in accordance with Art. 158(3) EPC

(43) Date of publication:
03.02.1999 Bulletin 1999/05

(21) Application number: **97902651.5**

(22) Date of filing: **12.02.1997**

(51) Int. Cl.[6]: **A61K 31/195**

(86) International application number:
PCT/JP97/00354

(87) International publication number:
WO 97/29744 (21.08.1997 Gazette 1997/36)

(84) Designated Contracting States:
AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE

(30) Priority: **15.02.1996 JP 65094/96**

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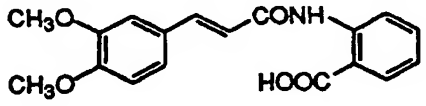
(54) NEOVASCULARIZATION INHIBITOR

(57) The present invention relates to a neovascularization inhibitor comprising as an active ingredient N-(3,4-dimethoxycinnamoyl) anthranilic acid represented by the formula:



or a pharmaceutically acceptable salt thereof, which has inhibitory effects on proliferation and chemotaxis of human microvascular endothelial cells and tube formation of human microvascular endothelial cells, and therefore, is useful as an agent for the prevention and treatment of diseases associated with neovascularization such as diabetic retinopathy, senile discoid macular degeneration, neovascular glaucoma and rheumatic arthritis.

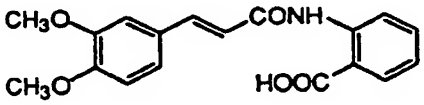
EP 0 894 496 A1

Description

Technical Field

[0001] The present invention relates to a pharmaceutical composition which is useful as a neovascularization inhibitor.

[0002] More particularly, the present invention relates to an agent for the prevention or treatment of diseases associated with neovascularization which comprises as an active ingredient N-(3,4-dimethoxycinnamoyl)anthranilic acid represented by the formula:



(I)

or a pharmaceutically acceptable salt thereof.

[0003] As diseases associated with neovascularization, various diseases which occur by participation of neovascularization as one of the causes,for example, diabetic retinopathy, senile discoid macular degeneration, retinopathy of prematurity, sickle-cell retinopathy, retinal vein occlusion, neovascularization after corneal transplantation or cataract extraction, neovascular glaucoma, rubeosis iridis, rheumatic arthritis, psoriasis, scleredema, tumors, overgrowth of capillary blood vessels in atherosclerosis adventitia and cornea] neovascularization caused by long wear of contact lens are examples.

Background Art

[0004] In general, neovascularization is a phenomenon accompanying degradation of the use membrane by proteolytic enzymes, chemotaxis and proliferation of endothelial cells, tube formation by endothelial cell differentiation and reorganization of blood vessels. Neovascularization occurs in luteinization and placentation physiologically and in the diseases mentioned above pathologically. For example, in retinopathy, retinal tissues lying between preexisting base membrane around retinal vessels and vitreous are degraded. Then, endothelial cells of preexisting vessels migrate from junctions of the degraded retinal tissues and endothelial cells proliferate to fill up spaces between the migrated endothelial cells, and the endothelial cells migrate to vitreoretina reorganize new vessels, leading to neovascularization.

[0005] Neovascularization is correlated with various diseases, and, for example, neovascularization plays a close role in the process of the onset and progress of the above diseases. Therefore, extensive studies to find compounds having an inhibitory activity on neovascularization have been actively promoted for the prevention or treatment of these diseases. Although, for example, neovascularization inhibitors such as fumagillin analogues, which are microbial metabolites having an inhibitory activity on endothelial cell proliferation, tetracycline antibiotics, which can inhibit collagenase activity, and microorganism-derived D-gluco-galactan sulfate, which can interfere with binding of heparin-binding angiogenic factors to their receptors, are known, there is no satisfactory drug clinically. In addition, there is no procedure adequate to treat the above diseases. Specially, if patients with diabetic retinopathy do not undergo surgical treatment, involution of neogenetic vessels cannot be observed, and therefore, visual loss caused by a discharge of blood from neogenetic vessels has become a problem. Thus, development of drugs having excellent effect on neovascularization are greatly desired.

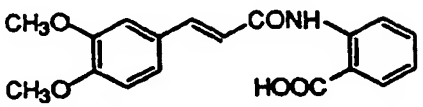
[0006] N-(3,4-Dimethoxycinnamoyl)anthranilic acid (generic name: Tranilast) represented by the above formula (I) of the present invention has been used widely as a drug for the treatment of allergic disorders such as bronchial asthma, allergic rhinitis, atopic dermatitis and allergic conjunctivitis, and cutaneous disorders such as keloid and hypertrophic scar. For example, it has been known that Tranilast has inhibitory activities on chemical mediator release caused by art allergic reaction, excessive collagen accumulation by fibroblast cells in cutaneous tissues and excessive proliferation of smooth muscle cells in coronary artery vessels.

[0007] However, it has in no way been disclosed that Tranilast suppresses proliferation and chemotaxis of microvascular endothelial cells and tube formation of microvascular endothelial cells, and it is not known at all that Tranilast is useful as a neovascularization inhibitor.

Disclosure of Invention

[0008] The present invention relates to a neovascularization inhibitor which comprises as an active ingredient N-(3,4-

dimethoxycinnamoyl) anthranilic acid represented by the formula:



(I)

or a pharmaceutically acceptable salt thereof.

[0009] The present invention relates to a method for the prevention and treatment of diseases associated with neovascularization which comprises administering N-(3,4-dimethoxycinnamoyl) anthranilic acid represented by the above formula (I) or a pharmaceutically acceptable salt thereof.

[0010] The present invention relates to a use of N-(3,4-dimethoxycinnamoyl)anthranilic acid represented by the above formula (I) or a pharmaceutically acceptable salt thereof for the manufacture of a pharmaceutical composition for the prevention and treatment of diseases associated with neovascularization.

[0011] Furthermore, the present invention relates to a use of N-(3,4-dimethoxycinnamoyl)anthranilic acid represented by the above formula (I) or a pharmaceutically acceptable salt thereof as a neovascularization inhibitor.

Brief Description of Drawings

[0012]

Figure 1 is a graph illustrating the inhibitory effect of Tranilast on proliferation of human microvascular endothelial cells. The axis of the ordinates shows the number of human microvascular endothelial cells (x 10^4 cells), and the axis of the abscissae shows concentrations of Tranilast (μg/ml). The symbols * and ** in the graph show the significant differences at $p<0.05$ and $p<0.01$, respectively.

Figure 2 is a graph illustrating the inhibitory effect of Tranilast on chemotaxis of human microvascular endothelial cells. The axis of the ordinates shows the number of human microvascular endothelial cells migrated (cells/visual field), and the axis of the abscissae shows concentrations of Tranilast (μg/ml). The symbol ** in the graph shows the significant differences at $p<0.01$.

Figure 3 is a graph illustrating the inhibitory effect of Tranilast on tube formation of human microvascular endothelial cells. The axis of the ordinates shows the number of networks of tubes formed (number), and the axis of the abscissae shows concentrations of Tranilast (μg/ml). The symbol ** in the graph shows the significant differences at $p<0.01$.

Figure 4 is a graph illustrating the inhibitory effect of Tranilast on tube formation of human microvascular endothelial cells. The axis of the ordinates shows the mean length of tube structure formed (mm), and the axis of the abscissae shows concentrations of Tranilast (μg/ml). The symbols * and ** in the graph show the significant differencesat $p<0.05$ and $p<0.01$, respectively.

Best Mode for Carrying Out the Invention

[0013] The present inventors have studied extensively to find compounds having inhibitory activity on neovascularization. As a result, it was found that N-(3,4-dimethoxycinnamoyl)anthranilic acid (generic name: Tranilast) represented by the above formula (I) has marked inhibitory effects on proliferation of human microvascular endothelial cells, chemotaxis of human microvascular endothelial cells and tube formation of human microvascular endothelial cells, and therefore, is extremely useful as a neovascularization inhibitor, thereby forming the basis of the present invention.

[0014] Accordingly, the present inventors confirmed that Tranilast significantly suppressed proliferation of human microvascular endothelial cells in the *in vitro* cell proliferation inhibitory activity test using human microvascular endothelial cells.

[0015] The present inventors also confirmed that Tranilast significantly suppressed chemotaxis of human microvascular endothelial cells in the *in vitro* cell chemotaxis inhibitory activity test using human microvascular endothelial cells.

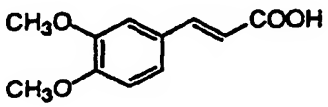
[0016] Furthermore, the present inventors also confirmed that Tranilast significantly inhibited tube formation of human microvascular endothelial cells in the *in vitro* cell tube formation inhibitory activity test using human microvascular endothelial cells.

[0017] As a consequence, Tranilast has excellent inhibitory effects on proliferation and chemotaxis in human microvascular endothelial cells, and therefore, is a compound which is useful as a neovascularization inhibitor. Furthermore, Tranilast has an excellent inhibitory effect on tube formation of human microvascular endothelial cells, and therefore, is

a compound which is extremely useful as an agent for the prevention and treatment of diseases associated with neovascularization.

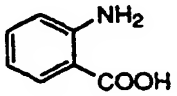
[0018] Therefore, pharmaceutical compositions which are useful as agents for the prevention and treatment of diseases associated with neovascularization can be prepared by including as an active ingredient Tranilast or a pharmaceutically acceptable salt thereof.

[0019] Various methods for the preparation of Tranilast and pharmaceutically acceptable salts thereof are known (Japanese Patent Application Publication (kokoku) No.Sho.56-40710; ibid. No.Sho.57-36905; ibid. No.Sho.58-17186; ibid. No.Sho.58-48545; ibid. No.Sho.58-55138; ibid. No.Sho.58-55139; ibid. No.Hei. 01-28013; ibid. No.Hei.01-50219; ibid. No.Hei.03-37539 etc.) For example, Tranilast and pharmaceutically acceptable salts thereof can be prepared by allowing a reactive functional derivative such as an acid halide and acid anhydride of 3,4-dimethoxycinnamoyl acid represented by the formula:



(II)

to react with anthranilic acid represented by the formula:



(III)

in the usual way, and if desired, converting the resulting compound into a salt.

[0020] As examples of pharmaceutically acceptable salts of Tranilast, salts with inorganic bases such as a sodium salt and a potassium salt, salts formed with organic amines such as morpholine, piperazine and pyrrolidine and salts formed with amino acids can be mentioned.

[0021] When the pharmaceutical compositions of the present invention are employed in practical treatment, various dosage forms of the pharmaceutical compositions can be used depending upon usage. As examples of such dosage forms, powders, granules, fine granules, dry syrups, tablets, capsules, ointments, injections, eye drops and the like can be given.

[0022] These pharmaceutical compositions can be formulated by admixing, diluting or dissolving, occasionally with appropriate pharmaceutical additives such as excipients, disintegrators, binders, lubricants, diluents, buffers, isotonicities, antiseptics, moistening agents, emulsifiers, dispersing agents, stabilizing agents and dissolving aids in accordance with conventional methods and formulating in the usual way depending upon the dosage forms.

[0023] For example, powders can be formulated by thoroughly mixing Tranilast represented by the formula (I) or a pharmaceutically acceptable salt with appropriate excipients, lubricants and the like as required.

[0024] Tablets can be formulated by admixing Tranilast or a pharmaceutically acceptable salt with appropriate excipients, disintegrators, binders, lubricants and the like as required, and pressing the mixture in the usual way. The tablets also can be coated to provide film-coated tablets, sugar-coated tablets, enteric coating tablets and the like.

[0025] For example, capsules can be formulated by thoroughly mixing appropriate excipients, lubricants and the like as required, and filling the mixture into capsules. Capsules can be also formulated by forming granules or fine granules in the usual way, and filling the granules or fine granules into capsules.

[0026] Ointments can be used as eye ointments.

[0027] Injections can be injected directly into diseased tissues such as cornea and vitreous or their adjacent tissues by using a fine needle, and can be also used as an intraocular perfusate.

[0028] The pharmaceutical compositions of the present invention can be administered as sustained release preparations. For example, Tranilast preparation is incorporated into a pellet or microcapsule of sustained release polymer as a sustained release preparation, and the pellet or microcapsule is surgically planted into tissues to be treated. As examples of sustained release polymers, ethylene-vinylacatate copolymer, polyhydromethacrylate, polyacrylamide, polyvinylpirrolidone, methylcellulose, lactic acid polymer, lactic acid-glycolic acid copolymer and the like can be mentioned, and preferably, a biodegradable polymer such as lactic acid polymer and lactic acid-glycolic acid copolymer can be used.

[0029] When the pharmaceutical compositions of the present invention are employed in a practical treatment, the dosage of Tranilast or a pharmaceutically acceptable salt thereof as the active ingredient is appropriately decided depending on the body weight, age, sex, degree of symptoms and the like of each patient to be treated, which is approximately

within the range of from 100 to 1,000 mg per day per adult human in the case of oral administration and approximately within the range of from 20 μg to 300 mg per day per adult human in the case of parenteral administration.

[0030] The dose of Tranilast or a pharmaceutically acceptable salt thereof can be appropriately increased and decreased depending on the type of diseases, severity of symptoms of each patient to be treated and therapeutic value.

Example

[0031] The present invention is further illustrated in more detail by way of the following Examples.

Study to confirm inhibitory effect on neovascularization

Example 1

Inhibition of proliferation of human microvascular endothelial cells

① Culture of human microvascular endothelial cells

[0032] Normal human dermal microvascular endothelial cells (Cell Systems Corporation) were subcultured in a medium (MVE medium, Cell Systems Corporation) for endothelial cell culture and used for the study. At the logarithmic growth phase, the medium was aspirated and cells were washed with phosphate-buffered saline (PBS(-)) which was added gently. Then, the PBS(-) was aspirated, an aliquot of 0.25% trypsin solution containing 0.02% EDTA was added to the culture plate, and the morphology of cells was observed under phase-contrast microscopy. When cells were going to be round, an equal value of MVE medium was added to the trypsin solution to stop the action of trypsin. Attached cells were harvested from the plate by pipetting the medium using a slender pasture pipette. Cell suspension was transferred into spit, then medium was added to the spit, and the cell suspension was mixed about 20 times vigorously by pipetting with a pasture pipett and centrifuged at 100-110xg for 1 minute. After the supernatant was discarded, fresh medium was added to the precipitate, and cell suspension was prepared by pipetting using a pasture pipett. Number of viable cells in an aliquot of the suspension was counted under a phase-contrast microscopy using a hemocytometer. Cell concentration was adjusted to 2×10^4 cells/ml.

② preparation of test drug

[0033] Tranilast was added to 1 % aqueous sodium bicarbonate solution to prepare 0.55 % solution and dissolved by warming at 70 °C. The solution was sterilized with millipore filter and diluted with MVE medium to a final prescribed concentration.

③ Experimental method

[0034] Cell suspension (1 ml) was added to collagen-coated 6-well plate(Toyobo Engineering Co., Ltd.) and cultured at 37 °C under a humidified atmosphere of 5 % CO_2 in air. After 1 day, the medium was aspirated, cells were washed with PBS (-), and 1 ml of fresh medium and 0.1 ml of various concentrations of Tranilast solution were added to the plate and the plate was incubated for further 2 days. After incubation, the medium was aspirated, cells were washed with PBS(-), and then 1 ml of 0.25 % trypsin solution containing 0.02% EDTA was added to the plate. After harvesting cells from the plate by pipetting using a pasture pipete, number of viable cells was counted using a hemocytometer.

④ Assessment of effect

[0035] Mean and standard variation value of each group were calculated. Statistical analysis of significance was performed by a one-way analysis of variance and statistical significance was confirmed. Thereafter, analysis of significance between groups was performed by Dunnett's multiple test.

⑤ Results

[0036] As shown in Figure 1, Tranilast significantly suppressed the proliferation of human microvascular endothelial cells in a concentration-dependent manner.

Example 2

Inhibition of chemotaxis of human microvascular endothelial cells

① Culture of human microvascular endothelial cells

[0037] According to the method of Example 1 ①, human microvascular endothelial cells were cultured to prepare a cell suspension. Number of viable cells was counted under a phase-contrast microscopy using a hemocytometer and cell concentration was adjusted to 2 x 10^4 cells/ml.

② Preparation of test drug

[0038] Tranilast was added to 1 % aqueous sodium bicarbonate solution to prepare 0.55 % solution and dissolved by warming at 70 °C. The solution was sterilized with millipore filter and diluted with DMEM + Ham (1 : 1) medium to a prescribed final concentration.

③ Experimental method

[0039] Chemotaxis of human dermal microvascular endothelial cells (prepared in ①) to vascular endothelial growth factor (VEGF) was studied using a 96-well micro chemotaxis chamber (Neuro Probe Inc.). An aliquot (32 µl) of DMEM + Ham (1:1) medium containing 100 ng/ml of VEGF, 0.1 % bovine serum and various concentrations of Tranilast was added to the lower cavity of the chemotaxis chamber. An aliquot (50 µl) of medium containing cell suspension and Tranilast was added to the upper cavity of the chamber. Polycarbonate filter (10 µm thickness with 8 µm pore size, Neuro Probe Inc.) coated with type-1 collagen was used for the chemotaxis membrane. The chemotaxis chamber was incubated at 37 °C for 5 hours under a humidified atmosphere of 5 % CO_2 in air. Cells migrated to the lower side of the filter were fixed with 90 % ethanol, and stained with Diff-Quick (Baxter Diagnostics Inc.). Number of migrated cells was counted in 5 random fields at x 400 magnification under a phase-contrast microscopy and mean chemotaxis cell number was calculated.

④ Assessment of effect

[0040] Mean and standard variation value of each group were calculated. Statistical analysis of significance was performed by a one-way analysis of variance and statistical significance was confirmed. Thereafter, analysis of significance between groups was performed by Dunnett's multiple test.

⑤ Results

[0041] As shown in Figure 2, Tranilast significantly suppressed the chemotaxis of human microvascular endothelial cells in a concentration-dependent manner.

Example 3

Tube formation of human microvascular endothelial cells

① Culture of human microvascular endothelial cells

[0042] According to the method of Example 1 ①, human microvascular endothelial cells were cultured to prepare a cell suspension. Number of viable cells was counted under a phase-contrast microscopy using a hemocytometer and cell concentration was adjusted to 4 x 10^4 cells/ml.

② Preparation of test drug

[0043] Tranilast was added to 1% aqueous sodium bicarbonate solution to prepare 0.5 % solution and dissolved by warming at 70 °C. The solution was sterilized with millipore filter and diluted with MVE medium to a prescribed final concentration.

③ Experimental method

[0044] An aliquot (0.25 ml) of matrigel (10 mg/ml, Becton Dickinson Labware) was added to a 24-well culture plate (Corning) and was then allowed to solidify by incubation at 37 °C for 1 hour. Suspension (0.25 ml) of human microvascular endothelial cells (4×10^4 cells) and MVE medium (0.25 ml) containing various concentrations of Tranilast were added onto the gel. After 18 hours incubation at 37 °C, 5 random fields of one well were observed at x 100 magnification using a phase-contrast microscopy and the number of formed networks was counted.

④ Result

[0045] As shown in Figure 3, number of the formed networks was significantly reduced in a concentration-dependent manner.

Example 4

Tube formation of human microvascular endothelial cells

① Culture of human microvascular endothelial cells

[0046] According to the method of Example 1 ①, human microvascular endothelial cells were cultured to prepare a cell suspension. Number of viable cells was counted under a phase-contrast microscopy using a hemocytometer and cell concentration was adjusted to 4×10^4 cells/ml.

② Preparation of test drug

[0047] Tranilast was added to 1% aqueous sodium bicarbonate solution to prepare 1.0 % solution and dissolved by warming at 70 °C. The solution was sterilized with millipore filter and diluted with MVE medium to a prescribed final concentration.

③ Experimental method

[0048] An aliquot (0.25 ml) of matrigel (10 mg/ml, Becton Dickinson Labware) was added to a 24-well culture plate (Corning) and was then allowed to solidify by incubation at 37 °C for 1 hour. Suspension (0.25 ml) of human microvascular endothelial cells (4×10^4 cells) and MVE medium (0.25 ml) containing various concentrations of Tranilast were added onto the gel. After 18 hours incubation at 37 °C, 5 random fields of one well were photographed at x 40 magnification using a phase-contrast microscopy. The lengths of the tube structures were measured and the mean value was calculated.

④ Result

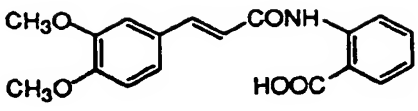
[0049] As shown in Figure 4, the length of the tube structure was significantly reduced in a concentration-dependent manner.

Industrial Applicability

[0050] A pharmaceutical composition comprising as an active ingredient Tranilast has remarked inhibitory effects on proliferation and chemotaxis of human microvascular endothelial cells and tube formation of human microvascular endothelial cells, and therefore, is extremely effective as a neovascularization inhibitor.

Claims

1. A neovascularization inhibitor which comprises as the active ingredient N-(3,4-dimethoxycinnamoyl)anthranilic acid represented by the formula:

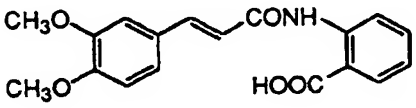


and a pharmaceutically acceptable salt thereof.

2. A neovascularization inhibitor as claimed in claim 1 wherein disease to be treated is diabetic retinopathy.

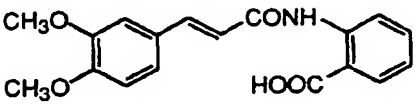
3. A neovascularization inhibitor as claimed in claim 1 wherein disease to be treated is senile discoid macular degeneration.

4. A method for the prevention and treatment of diseases associated with neovascularization which comprises administering N-(3,4-dimethoxycinnamoyl)anthranilic acid represented by the formula:



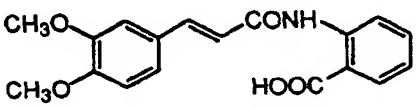
or a pharmaceutically acceptable salt thereof.

5. A use of N-(3,4-dimethoxycinnamoyl)anthranilic acid represented by the formula:



or a pharmaceutically acceptable salt thereof for the manufacture of a pharmaceutical composition for the prevention and treatment of diseases associated with neovascularization.

6. A use of N-(3,4-dimethoxycinnamoyl)anthranilic acid represented by the formula:



or a pharmaceutically acceptable salt thereof as a neovascularization inhibitor.

Figure 1

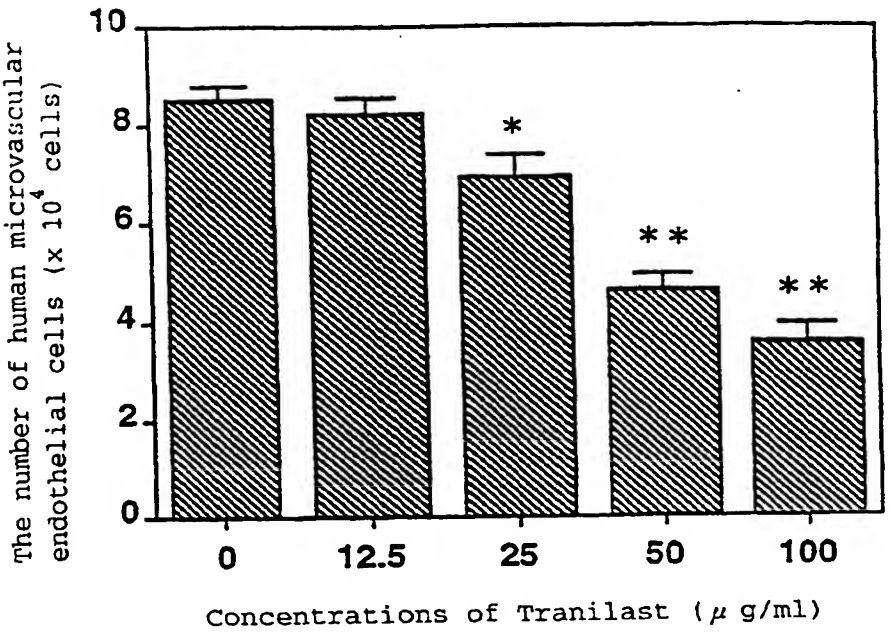


Figure 2

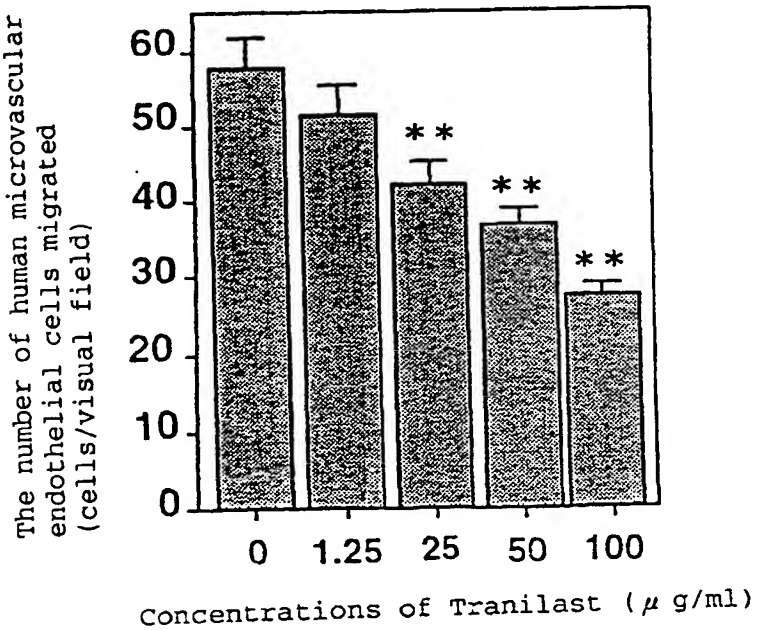


Figure 3

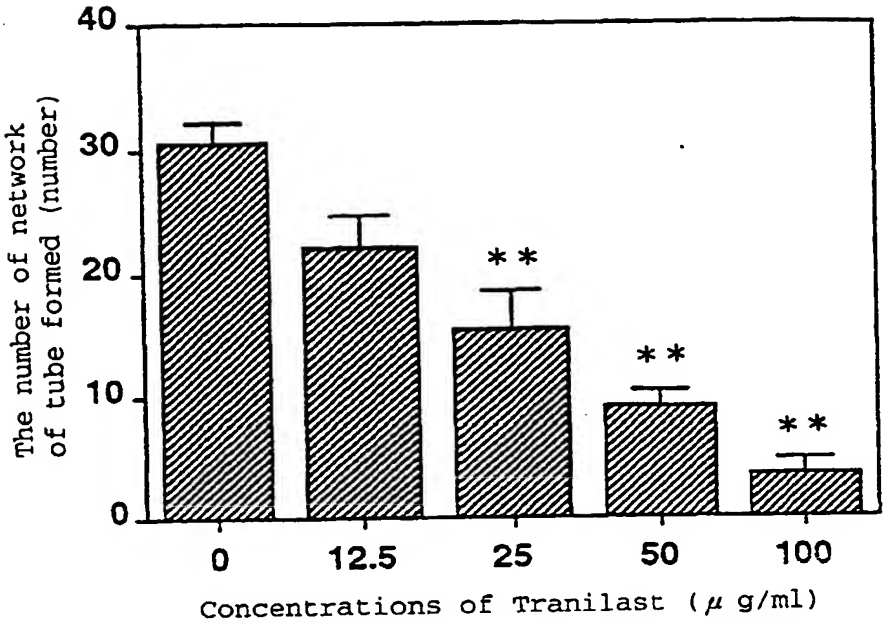
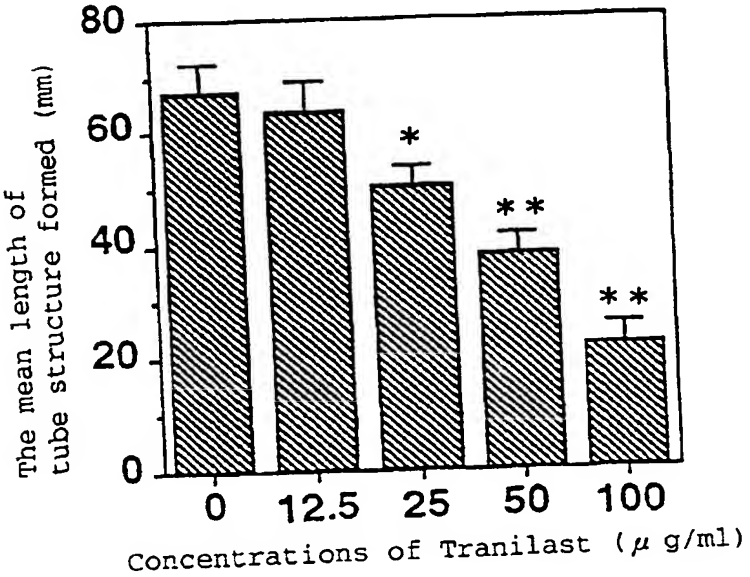


Figure 4



INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP97/00354

A. CLASSIFICATION OF SUBJECT MATTER
Int. Cl^6 A61K31/195
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
Int. Cl^6 A61K31/195, A61K31/00, A61K45/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JP, 5-163222, A (Kissei Pharmaceutical Co., Ltd.), June 29, 1993 (29. 06. 93), Particularly example 4 (Family: none)	1-3, 5
Y	FUKUYAMA, J. et al., "Inhibition effects of tranilast on proliferation, migration, and collagen synthesis of human vascular smooth muscle cells." Can. J. Phaysiol. Pharmacol., Jan. 1996, Vol. 74, No. 1. p. 80-84, especially, Abstract	1-3, 5
Y	MIYAZAWA, K. et al., "Inhibition of PDGF- and TGF-ß 1-induced collagen synthesis, migration and proliferation by tranilast in vascular smooth muscle cells from spontaneously hypertensive rats." Atherosclerosis, 1995, Vol. 118, No. 2, p. 213-221, especially, Abstract	1-3, 5
Y	TANAKA, K. et al., "Prominent inhibitory effects	1-3, 5

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
May 13, 1997 (13. 05. 97)	May 27, 1997 (27. 05. 97)
Name and mailing address of the ISA/ Japanese Patent Office	Authorized officer
Facsimile No.	Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP97/00354

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	of tranilast on migration and proliferation of and collagen synthesis by vascular smooth muscle cells." Atherosclerosis, 1994, Vol. 107, No. 2, p. 179-185, especially, Abstract	
Y	INGBER, D. et al., "Inhibition of angiogenesis through modulation of collagen metabolism." Laboratory Investigation, 1988, Vol. 59, No. 1, p. 44-51, especially, Abstract	1-3, 5
Y	MARAGOUDAKIS, M.E. et al., "Inhibition of basement membrane biosynthesis prevents angiogenesis." J. Pharmacol. Exp. Ther., 1988, Vol. 244, No. 2, p. 729-733, especially, Abstract	1-3, 5
Y	Moses, M.A. et al., "Isolation and characterization of an inhibitor of neovascularization from scapular chondrocytes." J. Cell Biol., 1992, Vol. 119, No. 2, p. 475-482, especially, Abstract	1-3, 5
Y	Takao Iwaguchi "New growth of blood vessel and control thereof (in Japanese)" Cancer & Chemotherapy 1993, Vol. 20, No. 1, pages 1 to 9, particularly, gist	2 - 3
Y	FRIEDLANDER, M. et al., "Definition of two angiogenic pathways by distinct αv integrins." Science, 1995, Vol. 270, No. 5241, p. 1500-1502, especially, left column of p. 1502	2 - 3
P,Y	FRIEDLANDER, M. et al., "Involvement of integrins $\alpha v\beta_3$ and $\alpha v\beta_5$ in ocular neovascular diseases." Proc. Natl. Acad. Sci. U.S.A., 1996, Vol. 193, p. 9764-9769, especially, Abstract	2 - 3
A	INGBER, D.E. et al., "A possible mechanis for inhibition of angiogenesis by angiostatic steroids: induction of capillary basement membrane dissolution." Endocrinology, Vol. 119, No. 4, p. 68-75	1-3, 5
A	JP, 6-135829, A (Kissei Pharmaceutical Co., Ltd.), May 17, 1994 (17. 05. 94) & EP, 588518, A & US, 5385935, A	1-3, 5
A	WO, 94/18967, A (Harvard College), September 1, 1994 (01. 09. 94) & JP, 8-506594, A & EP, 644760, A & US, 5512591, A	1-3, 5
A	KIKUCHI, S. et al., "Tranilast supress intimal hyperplasmia after photochemically induced	1-3, 5

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International application No.
PCT/JP97/00354

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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	endotherial injury in the rat." Eur. J. Pharmacol., Jan. 1996, Vol. 295, No. 2-3, p. 221-227	

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

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Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 4, 6
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 4 and 6 fall under the category of methods for treatment of the human or animal body by therapy and thus relate to a subject matter which this International Searching Authority is not required, under the provisions of Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

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